DEVELOPMENTAL MORPHOLOGY OF ASCOMYCETES

X. *NECTRIA HUMICOLA*

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SUMMARY. – Study of the developmental morphology of *Nectria humicola* Rama Rao and its anamorph. Discussion about its place among other genera of the Hypocreales.

RÉSUMÉ. – Étude de l'organogenèse de *Nectria humicola* Rama Rao et de son anamorphe appartenant au genre *Acremonium*. Comparaison avec *Nectria peziza* et *N. hematococca*.

MOTS CLEFS : Ascomycètes, Hypocréales, organogenèse, systématique.

This paper is the tenth in a series on the developmental morphology of Ascomycetes and deals with *Nectria humicola* Rama Rao. Our observations are based on a study of a fungus isolated from goat dung and made available by CHANDRASHEKARA (1977) who worked on coprophilous mycoflora in this Laboratory.

Nectria humicola was described by RAMA RAO (1969), for an isolate of a fungus from maize field soils from Narsapur, Andhra Pradesh, India. This fungus is characterized by 1) non stromatic perithecia which undergo a cupulate collapse when dry, 2) a perithecial wall which is pseudoparenchymatous and translucent except in the region of the perithecial papilla which is bright red in colour, 3) longitudinally striate ascospores and 4) an *Acremonium* state as anamorph. Because of these features, *Nectria humicola* belongs to the *Peziza* group of the genus *Nectria* of BOOTH (1959).

METHODS

For studying the various stages in the development of the anamorph and teleomorph, methods described earlier (SUBRAMANIAN & J. BHAT, 1978) were followed.

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DESCRIPTION OF THE FUNGUS

The fungus was grown on different media such as potato dextrose agar, potato carrot agar (PCA), malt extract agar and yeast extract soluble starch

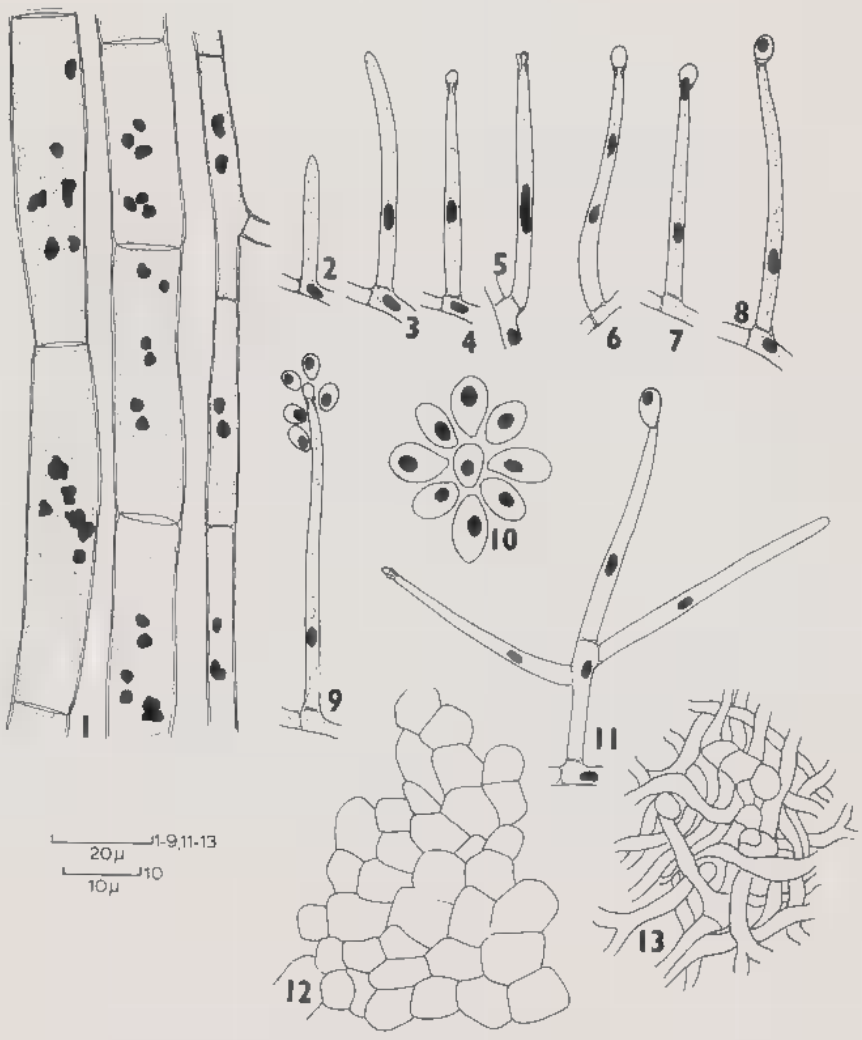


Fig. 1-13 : *Nectria humicola*. 1 : portions of vegetative hypha; 2-9 : stages in the development of phialide and conidium; 10 : conidia; 11 : a short phialophore bearing phialides; 12 : portion of perithecial wall (surface view); 13 : portion of prosenchymatous outer covering of the perithecial wall.

Fig. 1-13 : *Nectria humicola*. — 1 : fragments d'hyphe végétative; 2-9 : différentes étapes du développement des phialides et conidies; 10 : conidie; 11 : court phialophore portant des phialides; 12 : fragment de la paroi du périthèce (vu de surface); 13 : fragment de la couverture externe, prosenchymateuse, de la paroi du périthèce.

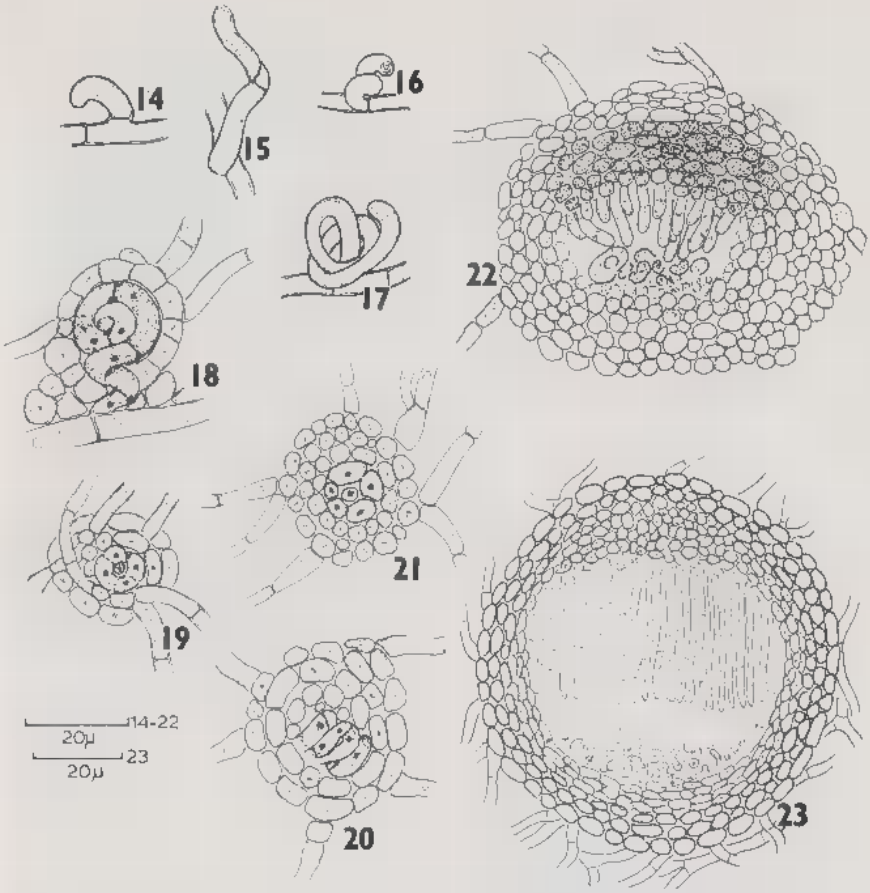


Fig. 14-23 : *Nectria humicola*. — 14-17 : stages in the development of ascogonium; 18 : ascogonium surrounded by hyphae; 19-21 : sections through coiled ascogonium; 22, 23 : sections through young perithecial centrum showing development of apical paraphyses.

Fig. 14-23 : *Nectria humicola*. — 14-17 : étapes du développement de l'ascogone; 18 : ascogone entouré par des hyphes; 19-21 : sections dans un ascogone enroulé; 22-23 : sections dans le centre d'un périthèce jeune montrant le développement de paraphyses apicales.

agar (YpSs) to find out the most suitable media for the production of both teleomorph and anamorph. In PCA and YpSs it produced both conidia and perithecia. In other media the conidial state alone developed. The following description is based on the fungus grown on YpSs medium.

Ascospores germinate whithin 12 hrs. producing a single germ tube terminally from each cell (Fig. 29). Colony 2.5 cm diam. in 12 days, floccose, circu-

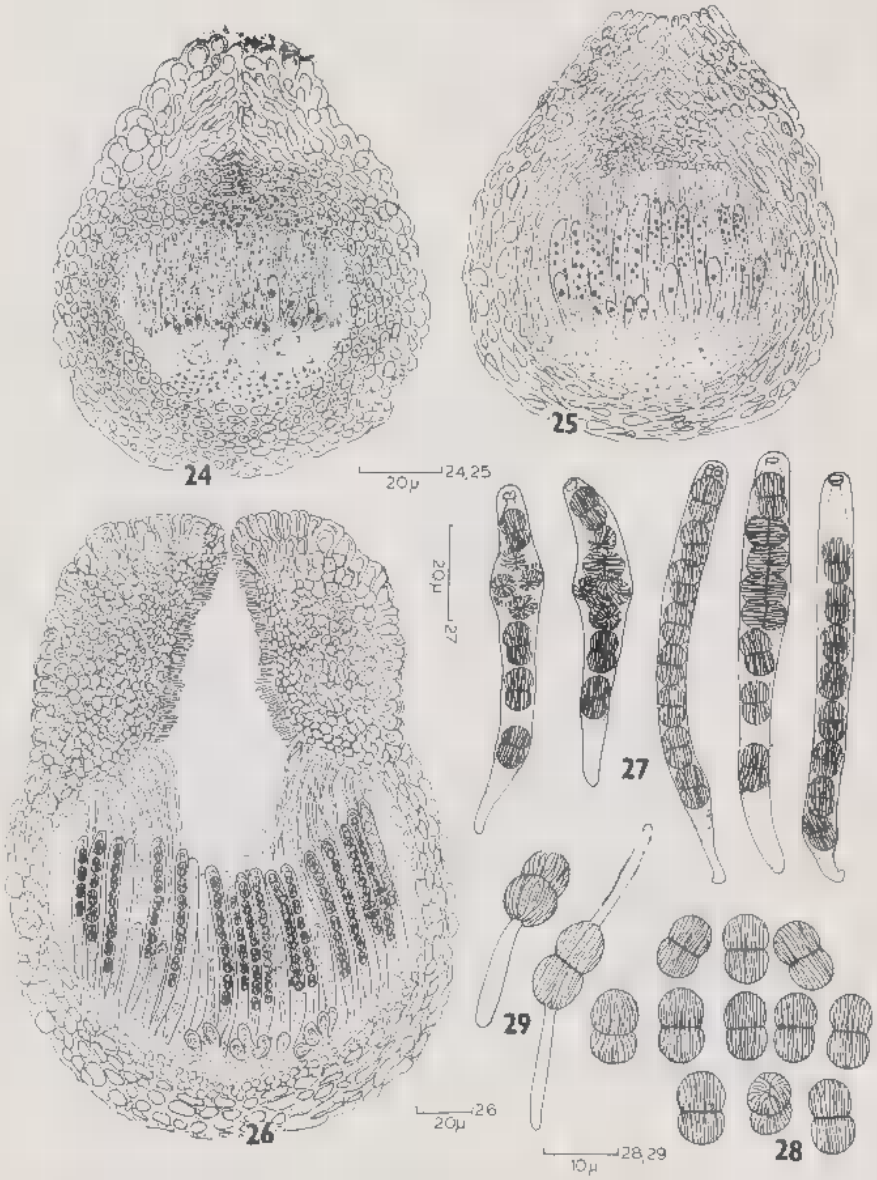


Fig. 24-29 : *Nectria humicola*. – 24, 25 : longitudinal sections of young perithecia showing apical paraphyses forming a palisade-like layer. Note in 25 the asci developing interspersed with apical paraphyses; 26 : longitudinal section of a mature perithecium; 27 : mature asci; 28 : ascospores; 29 : germinating ascospores.

Fig. 24-29 : *Nectria humicola*. – 24, 25 : sections longitudinales de jeune perithèce avec paraphyses apicales formant une couche d'aspect palissadique. On notera en 25 les asques se développant en mélange avec les paraphyses apicales; 26 : section longitudinale dans un périthèce mûr; 27 : asque mûr; 28 ascospores; 29 : germination d'ascospores.

lar, with surface white to buff coloured and reverse initially pale yellow and later becoming light brown. Mycelium slow growing, composed of hyaline, septate, branched, thick-walled hyphae with 2-multi-nucleate cells and up to 14.5 μm wide (Fig. 1; Plate I, e), producing phialides all over the surface of the colony. Phialides (Fig. 9, Plate I, b) mostly arising singly and laterally on vegetative hyphae, long, hyaline, cylindrical to subulate, thin-walled, smooth, narrowing towards the neck, with a cupulate collarette above the neck, producing a slimy mass of conidia at the tip, 24.0-52.0 μm long, 2.0-2.8 μm wide at the base and 1.2-1.8 μm at the neck; sometimes phialides arising in groups of 2-3 on short phialophores (20-30 x 3.5-4.5 μm) (Fig. 11). Conidia (Fig. 10; Plate I, c, d) 1-celled, solitary, hyaline, smooth, globose to obovate, with a basal apiculus, (3.9-11.7 x 2.6-3.8 μm).

Mature perithecia developing within 20 days of inoculation, solitary, partially immersed in the medium, hyaline to pale yellowish except fot the bright coloured perithecial papilla(Plate II, d), ovate to pyriform, smooth externally except for short protruding papillate cells in the neck region, ostiolate (220-330 x 140-220 μm). Perithecial wall pseudoparenchymatous; cells angular in surface view and 10-15 μm wide (Fig. 12), in longitudinal section 20-25 μm wide, consisting of 2 regions : an outer and an inner. Outer region 15-22 μm wide, composed of 2-4 layers of thick-walled, globose to elongate cells (5.0-6.0 x 6.0-9.0 μm). Inner region 10-12 μm wide, composed of 2-3 layers of thin-walled, elongated, compactly and parallely arranged cells. Outer region imperceptibly merging with inner region. Perithecial papilla 80-110 μm high, 90-130 μm wide, composed of thick-walled, globose, swollen cells towards the periphery and compactly arranged thin-walled smaller cells towards the interior. Ostiolar canal periphysate; periphyses (Fig. 50) slender, septate, with uninucleate cells (18-22 x 1.8-2.0 μm). Neighbouring vegetative hyphae enveloping perithecial wall and forming a prosenchymatous layer (Fig. 13). Young perithecium with a *Nectria*-type centrum. Apical paraphyses filamentous, slender, septate; cells swollen in the middle, 12.0-30.0 μm long, 5.0-11.0 μm wide and 2-10-nucleate (Fig. 51). Mature perithecium aparaphysate, undergoing cupulate collapse when dry.

Asci (Fig. 27; Plate II, e, f) unitunicate, typically cylindrical, often somewhat curved, truncate to broadly rounded at the apices, with distinct non-amyloid apical ring, 8- spored, (56-92 (75) x 6.2-7.0 μm). Ascospores (Fig. 28; Plate II, i, j) 1-septate, constricted at the septum, broadly rounded at both ends, hyaline to straw coloured, each cell with one nucleus and a large guttule, with prominent longitudinal striations in the epispore (8.2-13.0 (10.0) x 5.6-6.0 (5.5 μm); ascospores usually uniseriate in the ascus, but often some of the ascospores lying with their long axis at right angles to the axis of the ascus. Ascospores often liberated in a cirrus, sometimes the cirri sliming down and forming a slimy mass at the tip of the ostiole when dry spores appearing shining.

DEVELOPMENT OF THE ANAMORPH

As mentioned earlier, the fungus produces a phialidic conidial state which is referable to *Acremonium* Link ex Fr. The phialides are solitary and lateral

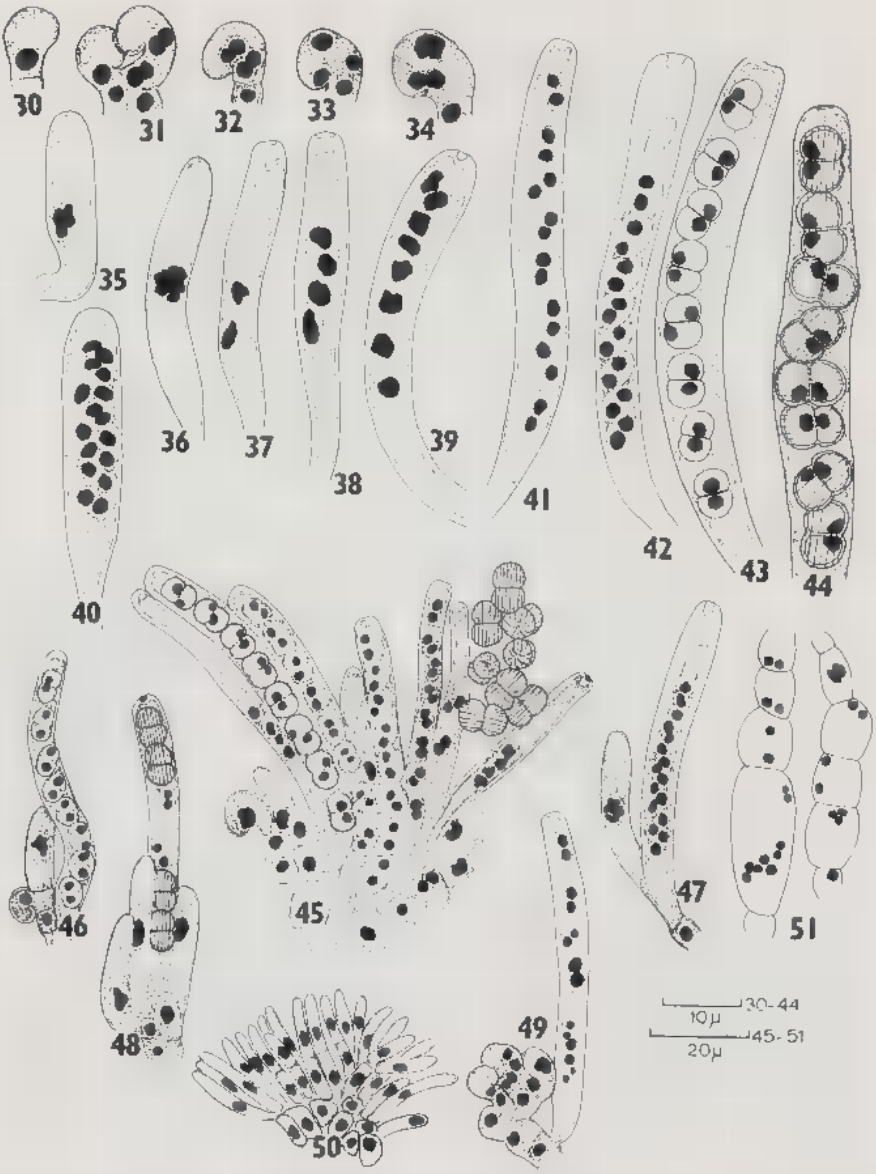


Fig. 30-51 : *Nectria humicola*. — 30-44 : stages in the development of ascus and ascospores; 45-49 : small clusters of asci. Note in 45, 46, and 49, croziers. Note in 48, only four ascospores in the ascus and in 49, the abnormal size of nuclei in the developing ascus; 50 : periphyses; 51 : portions of apical paraphyses with multinucleate cells.

Fig. 30-51 : *Nectria humicola*. — 30-44 : Différentes étapes du développement d'un asque et d'ascospores; 45-49 : petits bouquets d'asques; on notera les crochets en 45-46 et 49, seulement 4 ascospores en 48 et en 49, la taille anormale des noyaux dans l'asque en voie de développement; 50 : périphyses; 51 : fragments de périphyses apicales avec des cellules à plusieurs noyaux.

on the vegetative hyphae, long, subulate, uninucleate and separated from the parent hypha by a basal septum (Fig. 2, 3).

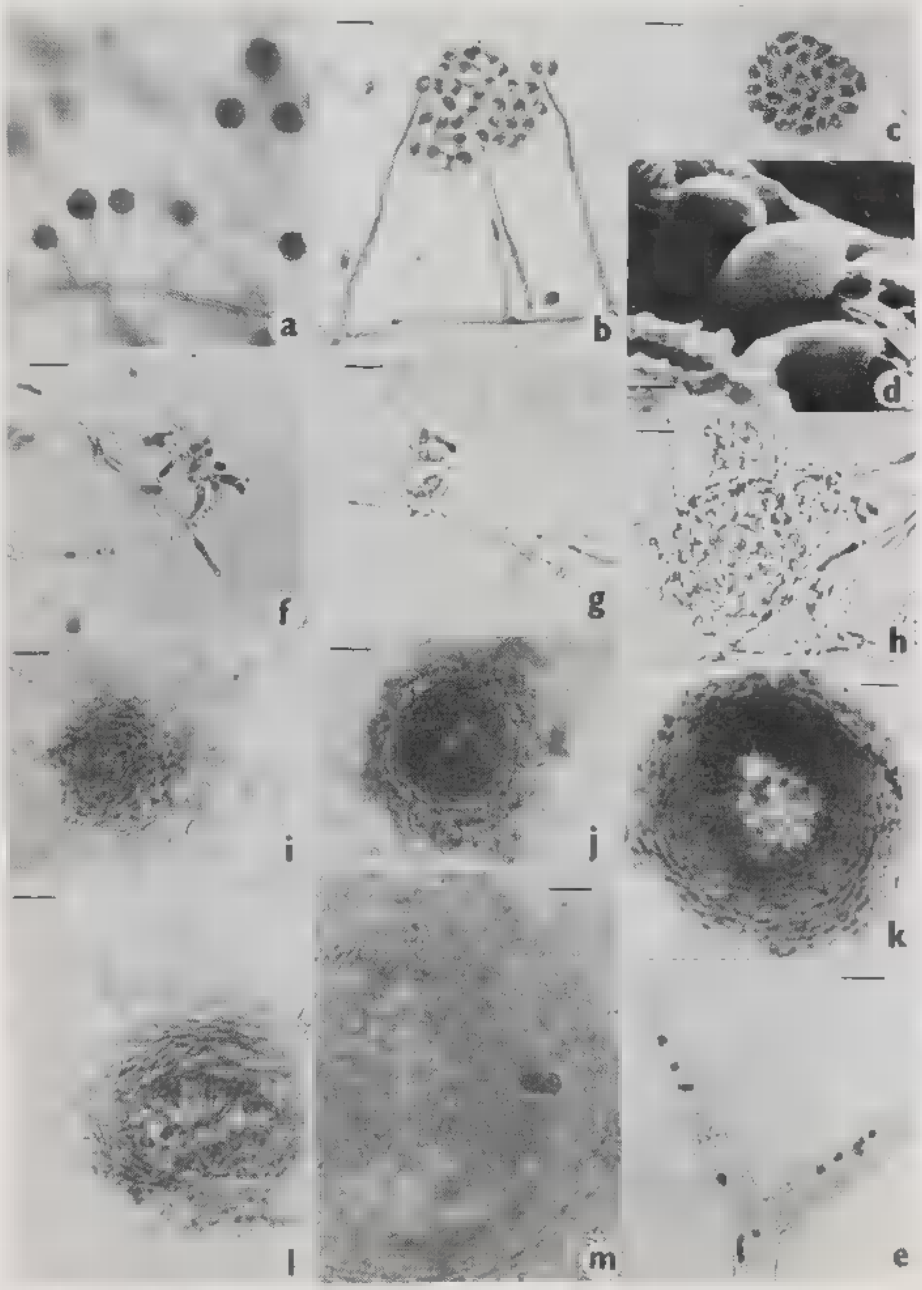
Early during the formation of a conidium, the apex of a phialide buds out a small protuberance (Fig. 4). In the meantime, the phialide nucleus elongates (Fig. 5) and becomes constricted in the middle and divides into two. The two daughter nuclei move apart (Fig. 6). The conidium initial increases in size and one of the daughter nuclei migrates into the conidium initial (Fig. 7). When the conidium attains its full size, a septum cuts off the conidium from the phialide (Fig. 8). As the mature conidium is detached from the phialide, a new conidium initial appears below it in the cupulate collarette (Fig. 9). This process is repeated. Often liberated conidia accumulate in a slimy mass at the phialide apex (Plate I, a).

DEVELOPMENT OF THE TELEOMORPH

The development of the perithecium is initiated by the formation of an ascogonium which arises as a lateral branch on the vegetative hypha and is solitary and slightly thick-walled (Fig. 14; Plate I, f). The ascogonium elongates and becomes coiled (Fig. 15-17; Plate I, g). Hyphae arising from the basal part of the ascogonial coil or from surrounding vegetative hyphae now grow and surround the coil (Plate I, h). With further development, the ascogonium becomes 4-8-celled, each cell being uninucleate (Fig. 18). The hyphae enveloping the ascogonium are relatively thin-walled, but contain dense cytoplasm (Fig. 19-21; Plate I, i). They proliferate further to form a compact plectenchymatous envelope (Plate I, j). The ascogonial cells divide into smaller cells and become multinucleate, presumably due to mitotic division of the nuclei as no migration of nucleus through anastomoses or otherwise is observed.

Further division of cells of the hyphal envelope increases the thickness of the envelope which becomes pseudoparenchymatous and is in fact the wall of the perithecium. In further development, a cavity develops around the ascogonial elements presumably due to disintegration of cells. The cells of the innermost layer of the perithecial wall lining the cavity now become «meristematic» and give rise to small angular cells. This «meristematic» activity, however, is more pronounced at the top and base of the cavity than on the sides of the inner layer of the wall. The angular cells elongate to form slender filaments with free tips which grow centripetally in the cavity (Plate I, k). Continued growth, however, is confined to filaments elongating downward from the roof of the cavity and these are the apical paraphyses (Fig. 22; Plate I, l, m). The growth of the apical paraphyses is not uniform initially, but later they develop synchronously (Fig. 23). The cells at the bottom of the cavity swell, take little stain and form a compact mass (Fig. 24).

The perithecium increases in size considerably as the apical paraphyses continue to develop. The ascogonial cells which have by now undergone further divisions form a layer at the base of the cavity. Apical paraphyses which continue to grow down now touch the bottom of the cavity (Fig. 24; Plate II, a).



At maturity, they are broad, septate, and with swollen multinucleate cells (Fig. 51). Asci grow up interspersed with apical paraphyses (Fig. 25). The pressure exerted by the developing asci contributes to the dissolution of the apical paraphyses (Fig. 26; Plate II, c).

As the apical paraphyses grow downward, the small angular cells formed at the apex grow upward and develop an ostiolar neck (Fig. 24, 25; Plate II, b). The cells constituting the ostiolar neck divide repeatedly and form a mass of deeply staining cells. In the meantime, an ostiole develops by dissolution of the cells in the core of the neck. In the mature perithecium, the cells lining the ostiolar canal produce slender filaments, the periphyses (Fig. 26).

DEVELOPMENT OF ASCI

Ascogonial cells are multinucleate with up to six nuclei. Each ascogonial cell puts out 2-3 short, stout, thick ascogenous hyphae (Fig. 30). The ascogenous hypha bends at its tip to form a crozier into which 2 nuclei migrate (Fig. 31; Plate II, g, h). Ascogenous hyphae are cut off from the ascogonial cells by the development of a septum at the base. In the meantime, the two nuclei undergo conjugate division to form 4 nuclei (Fig. 32). Two septa are laid down in the crozier separating an uninucleate tip cell and an uninucleate basal cell from a binucleate median cell (Fig. 33). The two nuclei in the median cell now fuse and form a diploid nucleus (Fig. 34). The median cell (ascus initial) elongates and the fusion nucleus moves to the middle of the initial (Fig. 35). The ascus initial is cylindrical and is rounded at the tip. At this stage, the nucleus passes through stages of first meiotic division; five chromosomes are visible at early prophase stage (Fig. 36, 47). Following first meiotic division two nuclei are formed (Fig. 37) and second meiotic division results in 4 haploid nuclei (Fig. 38). A third division which is mitotic immediately follows, resulting in 8 haploid nuclei (Fig. 39). Each nucleus in the 8 nucleate ascus simultaneously undergoes a fourth (mitotic) division resulting in eight paired nuclei (Fig. 40, 41). The

Plate I : *Nectria humicola*. – a: phialides with slimy conidial heads; b : a few phialides and uninucleate conidia; c : uninucleate conidia; d : microconidia under SEM; f, g : stages in the development of ascogonium; h : ascogonium surrounded by an envelope of hyphae; i, j : sections showing coiled ascogonium surrounded by envelope of hyphae; k : section of a young perithecial centrum showing centripetally growing hyphal processes in the perithecial cavity; l, m : sections of perithecial centrum showing apical paraphyses; e : portion of a vegetative hypha showing multinucleate cells.
Scales : 10 µm, d : 1 µm.

Planche I : *Nectria humicola*. – a : phialide avec une tête conidienne muqueuse; b : phialides avec conidies uninucléées; c : conidies uninucléées; d : microconidies vues au MEB; f, g : différentes étapes du développement de l'ascogone; h : ascogone entouré par des hyphes; i, j : coupes montrant l'ascogone enroulé entouré par des hyphes; k : coupe dans le centre d'un jeune périthèce montrant la croissance centripète des hyphes dans la cavité périthéciale; l, m : coupes dans le centre d'un périthèce montrant les paraphyses apicales; e : fragment d'hyphe végétative à cellules plurinucléées.
Échelles : 10 µm, d : 1 µm.

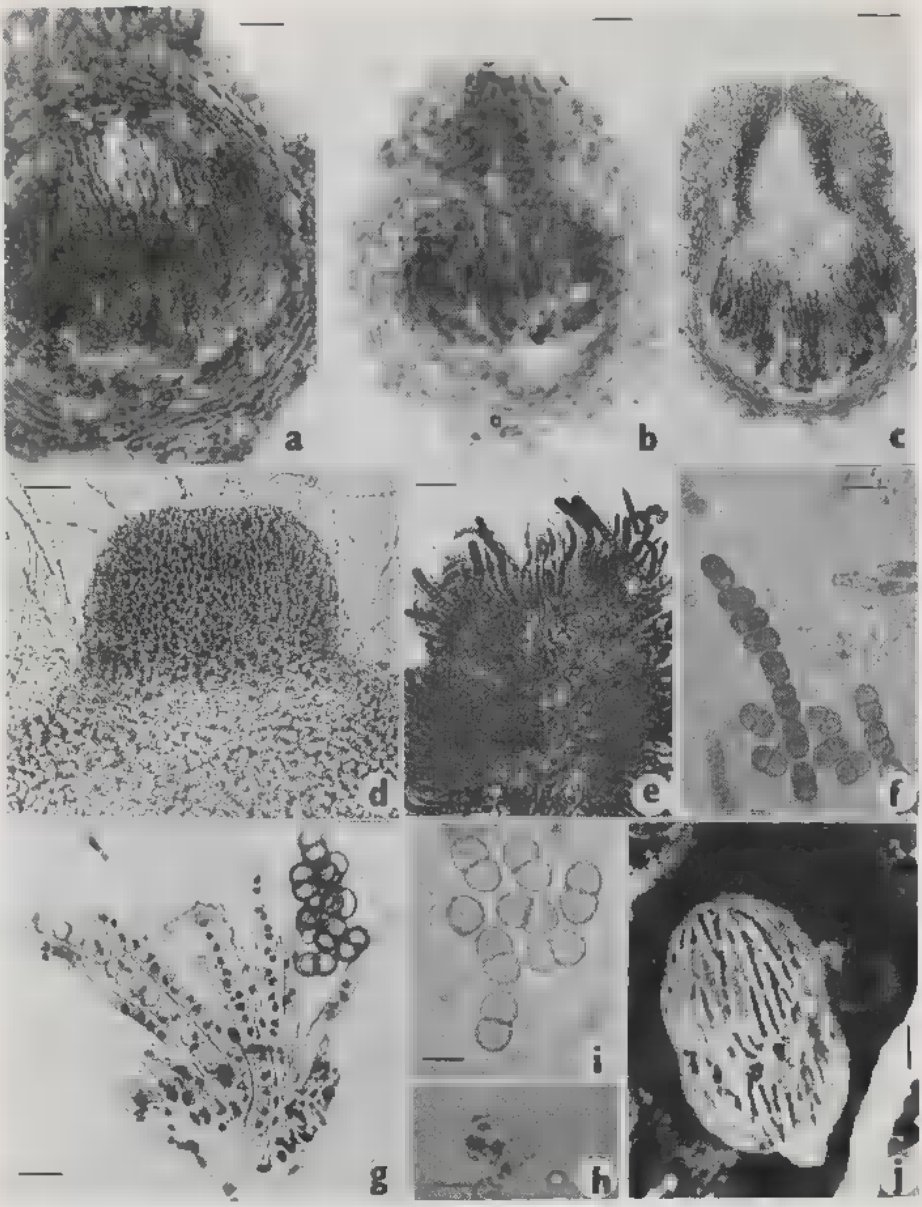


Plate II : *Nectria humicola*. — a, b : sections of young perithecia showing developing asci interspersed with apical paraphyses ; c : section of a mature perithecium; d : perithecial papilla (whole mount); e : cluster of asci; f : an ascus with 8 ascospores; g : a cluster of asci showing different stages in development of asci; h : a crozier; i : ascospores; j : ascospores under SEM showing striations.
Scales : a, b, c, d : 30 µm; e, f, g, h, i : 10 µm; j : 40,5 µm.

plane of this division probably decides the final spore arrangement in the ascus. Cleavage of the cytoplasm takes place in such a way as to produce elliptical masses around each pair of nuclei. A cell wall is laid down around each elliptical mass to produce eight young ascospores (Fig. 42). Each ascospore now develops a median septum and becomes 2-celled ascospores (Fig. 43). Longitudinal striations appear in the epispore when the spores are nearly mature (Fig. 44).

Often the tip cell and the basal cell fuse and give rise to another ascus. Up to three asci were observed in some ascogenous hyphae (Fig. 45).

A distinct non-amyloid ring is visible at the tip of the ascus even at the two-nucleate stage of the developing ascus. This becomes distinct after the 8-nucleate stage in the development of the ascus.

At times, nuclear behaviour during the development of the ascus may show some irregularities. For example, some of the asci have only four mature ascospores, the remaining four nuclei being abortive (Fig. 48, 49).

DISCUSSION

In *Nectria humicola* the conidial state is an *Acremonium*. The first step in the development of the perithecium is the formation of a coiled, septate ascogonium, the cells of which initially are uninucleate, and later become multinucleate. Although perithecial centrum is essentially of the *Nectria*-type (LUTTRELL, 1951) cylindrical to subcylindrical simple hyphal processes grow towards the centre of the perithecial cavity from all over the surface of the innermost layer of the perithecial wall. The growth of these processes possibly serves the function of creating a sizeable cavity in the perithecial centrum since they eventually disappear and then the apical paraphyses develop and grow down from the roof of the cavity. The presence of finger-like processes seems to be noteworthy in *N. humicola* which otherwise shows features conforming to the *Nectria*-type centrum described by LUTTRELL. It is significant that the occurrence of similar processes growing centripetally from all over the inner surface of the perithecial cavity is also illustrated by VINCENS (1917, fig. 40) for *Hypocrea gelatinosa*, by HANLIN for *Hypocrea schweinitzii* (HANLIN, 1965, fig. 16-17) and *Nectria haematococca* (HANLIN, 1971, fig. 30) and, in addition, described by CANHAM (1969) for *Hypocrea citrina* and by SAMUELS (1973) for *Hypomyces aurantius*. Thus, the presence of centripetally growing processes is now known in three genera of the Hypocreales, namely *Hypocrea*, *Hypomyces* and *Nectria* although only some but not all species of these genera show this feature.

Pl. II : *Nectria humicola*. — a, b : coupes dans de jeunes périthèces montrant des asques imbriqués dans des paraphyses apicales; c : coupe dans un périthèce mûr; d : papille périthéciale; e : bouquet d'asques; f : asques avec 8 ascospores; g : bouquet d'asques montrant différentes étapes de développement; h : crochet; i : ascospores; j : ascospores vues au MEB montrant des striations.

Échelles : a, b, c, d : 30 µm; e, f, g, h, i : 10 µm; j : 40,5 µm.

Perithecial wall in *Nectria humicola*, in the outer region composed of thick-walled, rounded to elongated cells which imperceptibly merged into the inner region of similar but somewhat thin-walled cells. Asci of *Nectria humicola* have an apical non-amyloid ring, a character it shares with many hypocrealean taxa (CHADEFAUD, 1961).

From a study of *Nectria peziza*, MUNK (1957) suggested that like *N. peziza*, other species with striate ascospores may also have a pseudoparenchymatous centrum. It is on the strength of this suggested correlation that MUNK established the genus *Neuronectria* for *Nectria peziza* (Tode ex Fr.). BOOTH (1959) who studied the authentic exsiccata of *Nectria peziza* disagreed with MUNK's transfer of the fungus into a new genus because the striate ascospores were found in species of *Nectria* with apical paraphyses.

HANLIN's study of the developmental morphology of *Nectria peziza*, *N. haematococca* and the author's study of *Nectria humicola* have shown that these 3 species essentially are *Nectria*-type sensu LUTTRELL, having apical paraphyses, notwithstanding the fact that these have striate ascospores. Therefore, MUNK's suggested correlation does not hold and the genus *Neuronectria* is superflous.

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